Global Scientific JOURNALS

GSJ: Volume 9, Issue 2, February 2021, Online: ISSN 2320-9186
www.globalscientificjournal.com

Different types of PCR

A review paper

Mohammad Ibrahim Khalil

Environmental Science and Technology College
University of Mosul
mohammadibrahim@uomosul.edu.iq

Abstract

Polymerase chain reaction (PCR) a technique which open a new level of scientific research in different direction to understanding the source of some genetics pathogen and polymorphism among population, its offer to amplifying the DNA outside of the organism " in vitro". A different type of PCR were created for detection many phenomena which appear in different disease and cases, abled the scientist to study these cases and diagnosis the reasons. RAPD, AFLP, ARMS, Colony, Nested and multiplex and other types of PCR are reveal the reasons of many genetics and heredity of some trait and disease.

Polymerase Chain Reaction (PCR)

Invented the PCR by the scientist Kary Mullis in 1983, he won the Nobel Prize and the Japan Science Prize for this invention, and it relies on basic biochemistry to amplified a small piece or pieces of DNA. DNA

can enzymatically duplicate to a millions copy outside living systems on a logarithmic or incremental scale with primers and in a short time, the goal is to amplify a specific part of the DNA that usually contains useful diagnostic or therapeutic information, (1).

DNA amplification takes place after the reaction mixture for PCR which is consist of the DNA template to be replicated and the main mixture, which is also consists of the primers (small DNA nucleotides of four nitrogenous bases and the presence of the enzyme DNA polymerase thermally stable and magnesium chloride, as well as nuclease free water eppendorf tube, (2).

The classic PCR including three series iterative stages that form the basis of the chain reaction:

Denaturation stage :

This stage starts by raising the temperature of the mixture 94 °C - 96 °C to separate the two strands of DNA from each other, in which the double chain is transformed into a single chain, and it occurs in a period of time ranging between 1-9 minutes, as the two chains are physically separated from each other due to the breakage of the hydrogen bonds that bind them at high temperatures. It is worth noting that the most common causes of failure of interaction PCR; It is the incomplete deformation of the DNA molecule, so this high temperature is used to ensure complete deformation. Thermal metamorphosis is preferred over chemical as it can be easily reversed by cryotherapy, (3).

Primer annealing:

This stage begins when the reaction mixture is cooled and associated with primers that surround the region to be amplified with template of DNA ;These primers are short of single-stranded nucleotides made in the laboratory, and are complementary to DNA; The original template to be duplicated. The primers are usually consist of 12-20 nucleotides in length

and are either special or unique precursors (unique) for a specific sequence of the DNA ; or they may be general (universal) for conserve region in DNA. This stage occurs at a temperature lower or equal to the melting temperature (55-68) ° C; the two single primers forward and reverse combined with the DNA template in two direction, the forward primer anneal from 3` to 5` strand (antisense) whereas from 5` to 3` (sense) strand, and building the hydrogen bonds between them, takes approximately 10 to 60 seconds, and the temperature adopted in this step depends on the constituent bases of the initiator as well as its length and the percentage of its containment of bases G and C,(4) .

The elongation (Extended) stage:

This is the last stage of the PCR process and it occurs at a temperature of 80-72 ° C, and this temperature is optimum for DNA polymerase enzyme activation by building a new DNA strand, and this stage begins with the help of the enzyme polymerase by adding nitrogenous bases in the direction 5` → 3` , and a reaction can be applied PCR on DNA and RNA, for RNA Perform an initial step which is creating a complementary version of Complementary DNA (cDNA) By reverse transcription enzyme (RT) reverse transcriptase ,to be subjected after cDNA this technique is known as previous amplification stages RT - PCR (Al-Samurai, 2018), fig (1).

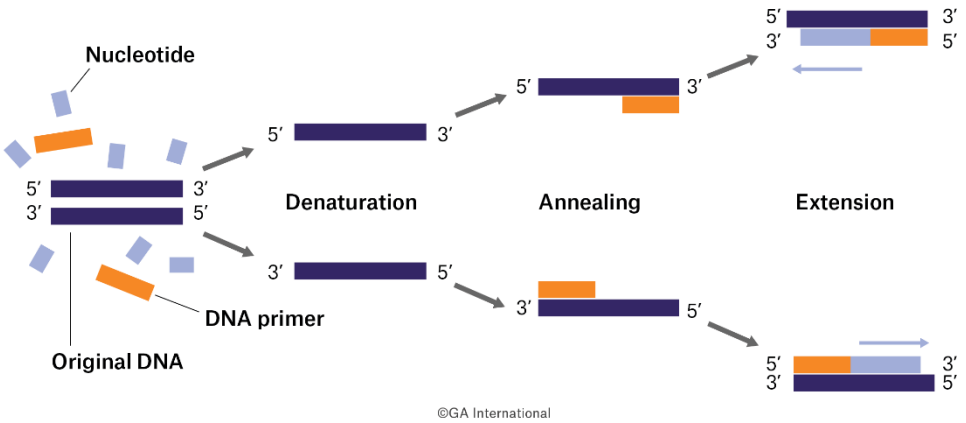

Figure (1): PCR steps

Multiplex PCR

Multiplex PCR is a widely used molecular biology technique for amplifying multiple targets in a single PCR experiment. In a multiplexing test, more than one target sequence can be amplified using multiple initiator pairs in a reaction mixture. As an extension of the practical use of PCR, this technique has the potential to achieve significant savings in time and effort within the laboratory without compromising the usefulness of the experiment, (5).

Types of Multiplex PCR

Multiplication reactions can be broadly divided into two categories:

1. One template PCR reaction

This technique uses a single template that can be genomic DNA with multiple pairs of forward and reverse primers to amplify specific regions within the template.

2. Multiple PCR template reaction

Uses multiple templates and several primer sets in the same reaction tube. The presence of multiple DNA template may cross-hybridize with each other and potentially mislead with other DNA template.

Multiple PCR reaction to amplify multiple target sequences in a single reaction tube, fig (2).

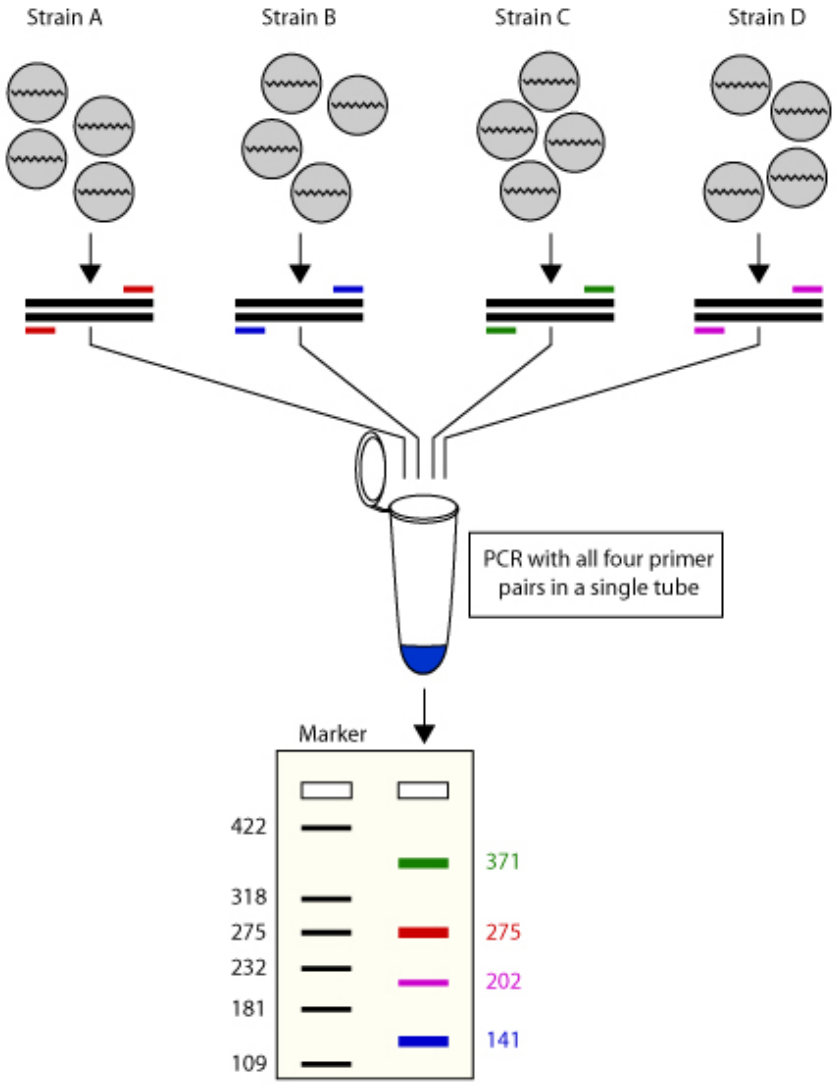

Figure (2): Multiplex PCR

Nested PCR

This type of PCR is used to increase the specify of PCR to amplify the required region in DNA template and to eliminate the non-specific amplified region with other two primers. Two set of primer were used in this type of PCR, the first primer set of primer is binds in different site (outside of DNA target) and amplified larger size of DNA, this set is noun outer primes, the other set is binding specifically to required target site. The second set is followed the first set and only amplified the target site within the first amplified region by first set of prime. The aim of this reaction " nested PCR" is to degrease the non- specific amplification which elimination the undesired region by fist set of primer. this type of

PCR is similar of traditional PCR except one more set of primer and decrease the non-specific binding site because the template of this reaction is the product of amplification of the first set of primer, (6), fig (3).

Colony PCR

Colony PCR is a technique which used to detection of the present or absent DNA segment which inserted into bacterial plasmid colony directly, it's a novel technique depends on designing a specific primer to amplify the inserted DNA to the plasmid. Two types of primer can use to detection the inserted DNA, one is used to amplify the inserted DNA and the other to amplify the both of the DNA and the plasmid. In this technique a single colony was picked and directly added to PCR tube which represent the DNA template with all PCR reaction components, its differ from traditional PCR by one more step; heating the colony to release the plasmid from the bacterial cells. The protocol didn't need to DNA extraction but need to pick one colony by tooth-stick and transfer it to eppendorf tube, re-suspend the colony by TE buffer, then the suspension heating by using boiling water for 20 minutes and the centrifuge at high speed for 2 minutes, the supernatant used as DNA template, (7), fig (4).

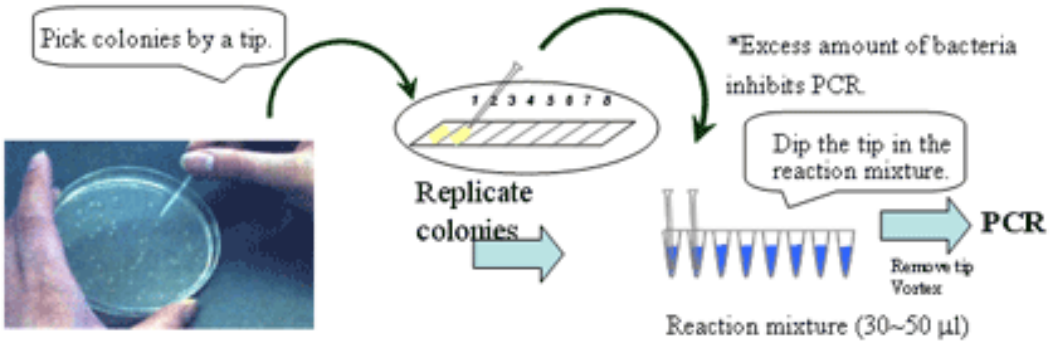

Figure (4): Colony PCR

RAPD PCR

Random Amplification polymorphism DNA (RAP) PCR is another PCR technique, the main specification of this reaction is doesn't need a specific primer to amplification the DNA template, instead using a short

oligonucleotide "10- mer" primer to amplify or not the DNA template. In this type of PCR; usually used more than one primer. The result depends on numbers of present or absent bands on agarose gel after PCR, which represent the polymorphism of the DNA as a result of mutation or any changing in sequences of the DNA. Theses polymorphisms can be represented by dendrogram or phylogeny tree of the organism DNA under different condition " similarity and distance", (8), fig (5).

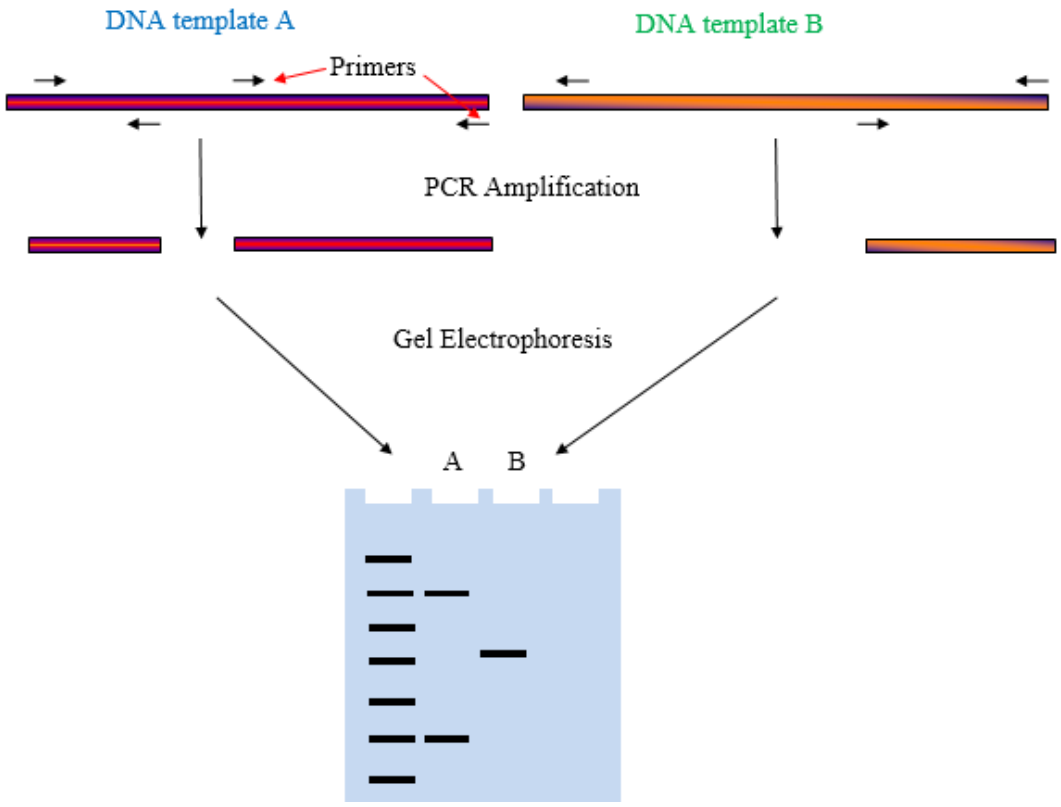

Figure (5): RAPD PCR PCR

SSR-PCR:

It’s an abbreviation of " Simple Sequence repeat anchored polymerase chain reaction"; it’s a technique depend on sequences of primer on the 5` or 3` end of microsatellite " a short repeated sequences of DNA at specific one locus of a chromosome used as fingerprinting in genetics" the nucleotide typically repeated 5-50 times. The primer used a highly annealing temperature. The microsatellite is a highly polymorphic mutation and distribution in eukaryotic genomic DNA, it's not exceed 200 bp which targeting amplifying more one locus in microsatellite DNA, (9), fig (6).

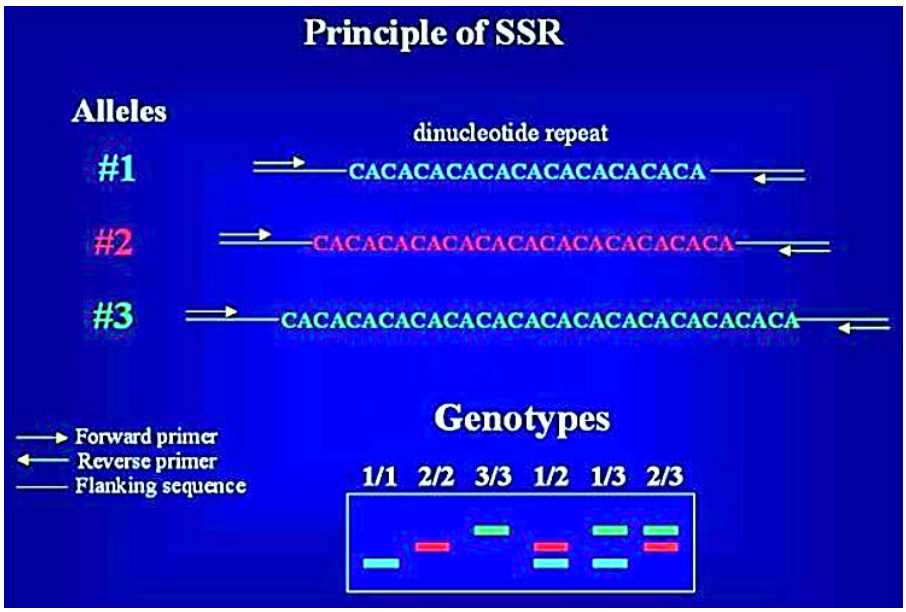

Figure (6): SSR PCR

AFLP and RFLP PCR

AFLP PCR " Amplified Fragment Length Polymorphism " it's used a restriction enzyme to digest the DNA then amplified the digested segment by PCR, this reaction is used to detect the polymorphisms of fingerprinting with related population. While RFLP-PCR " Restriction fragment length Polymorphisms" is used the restriction enzymes to digest the genomic DNA then running the gel electrophoresis and making the blotting by using radioactive labeled probe, (10), fig (7).

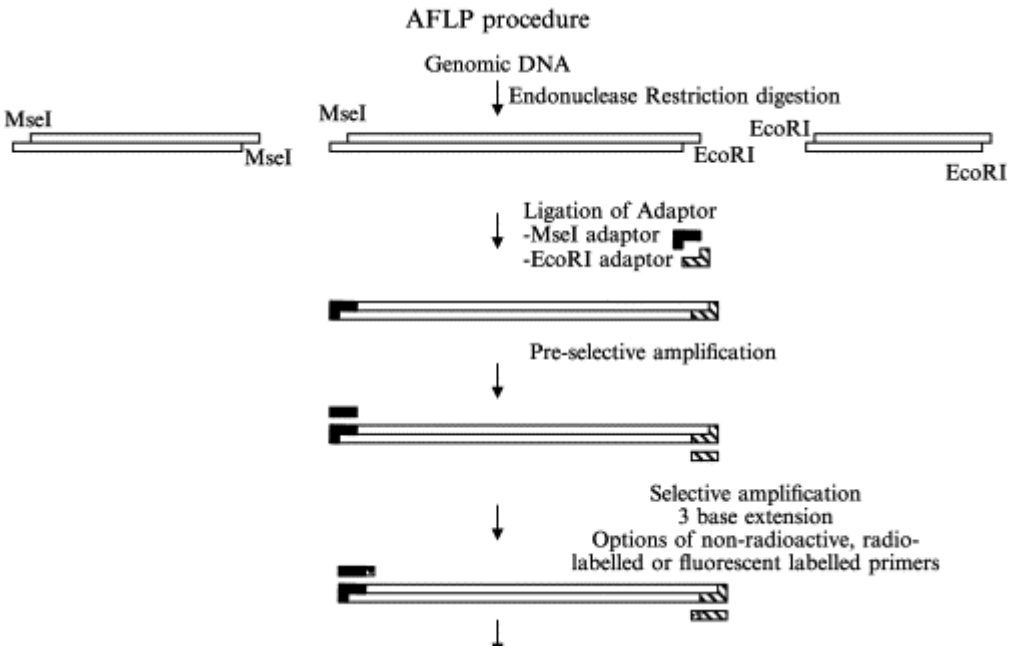

Figure (7): AFLP PCR

ARMS PCR

It's an abbreviation of " Amplification of Refractory Mutation System" which is a technique used to detect a single nucleotide change in the sequence, it's also called " PCR Amplification of specific alleles" or " single nucleotide Polymerase", its used to detect known mutation but not new mutation, its depends on designing a specific primer for two alleles; which is one normal and the other is polymorphisms in single nucleotide.

The two primers are, fig (8), one matches the natural allele and the other match the abnormal allele (mutant) and that is depends on one base in 3` end. In primer design; a modification of primer must be considering to achieve successfully results. At the near 3` end (2 base before the end) of primer a strong mismatch base is added, (C: T, G: A, T: T), this mismatch will alter the annealing temperature, if the template has a complementary base (normal allele) the amplification will have done with presence the strong mismatch, while if there is a non-complementary base in the 3` end of primer (mutant allele) the amplification will terminate,(11), fig (9).

Figure (8): Designing ARMA PCR

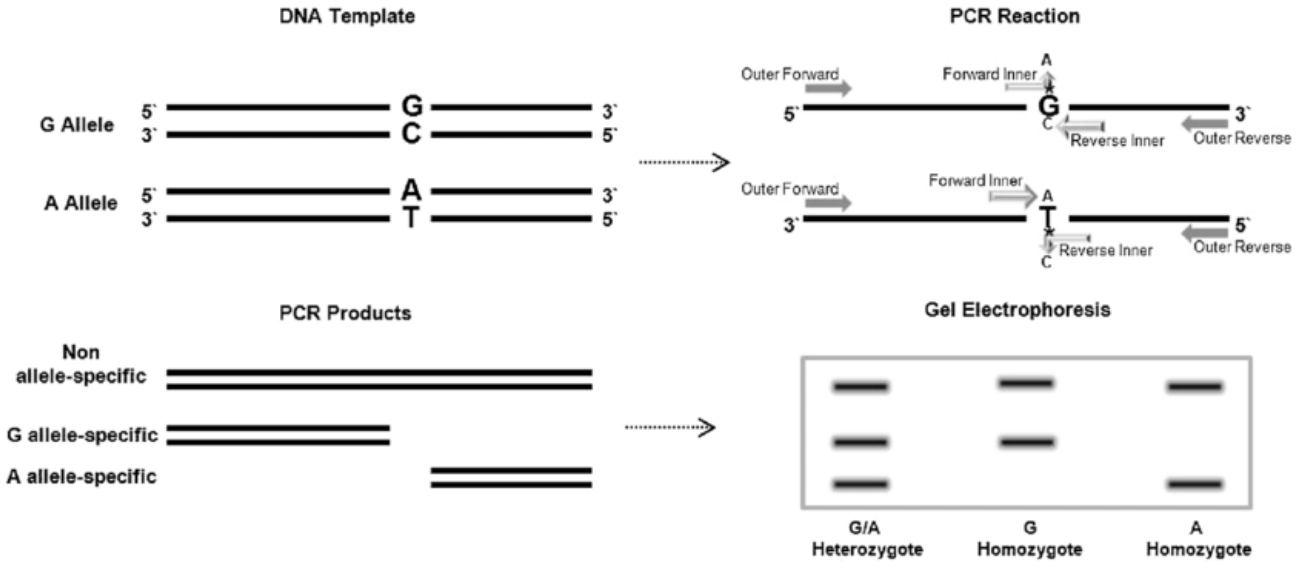

Figure (9): ARMA PCR

References

(1). Laura Doyle, Sherilynn Vogel, Gary W Procop, *Pneumocystis* PCR: It Is Time to Make PCR the Test of Choice, *Open Forum Infectious Diseases*, Volume 4, Issue 4, Fall 2017, ofx193,

(2). Khalil, Mohammad I. , Jaffar, S. and Yousif, B. (2018). Molecular Detecting of fungi and Bacteria in the Blood of Patients With Genital System Inflammatory Infection, Journal of Babylon University/Pure and Applied Sciences/ No.(1), Vol.(26).

(3). Khalil, M. I.(2020).CRISPR/Cas9 design to knockout and knockin the breast cancer gene- BRCA1 in Arabidopsis thaliana, International Conference on Pure Science (ISCPS-2020), Journal of Physics: Conference Series.

(4). Khalil, M. I. (2020).in silico detection of dna methylation in fungi neurospora crassa genes, rid-1 and dim-2, conference: 2nd international conference on materials engineering & science, aip conference proceedings 2213(1):020072

(5). Khalil, M. I. (2020).identification of cladosporium sp. fungi by in-silico rflp-pcr, baghdad science journal 17(1(suppl.)):0220

(6). Khalil, M. I. (2020).effect of smoking on dna methylation by using neurospora crassa fungi as a model, biochemical and cellular archives 20 (2):5575-5580.

(7). Karim Kadri (2019). Polymerase Chain Reaction (PCR): Principle and Applications, Synthetic Biology - New Interdisciplinary Science.

(8). Maria D, Angela P, Chialexei L (2008) Characteristics of RAPD markers inbreeding of Cucumis sativus L. Roum Biotech Lett 13:3843–3850

(9). Livak KJ and Schmittgen TD (2001). Analysis of relative geneexpression data using real-time quantitative PCR and the 2-Ct method. Methods 25: 402-408

(10). Hayden, M.J., T.M., Nguyen, A., Waterman andChalmers, K.J. 2008. Multiplex-ready PCR: Anew method for multiplexed SSR and SNPgenotyping. BMC Genomics. 9:80.

(11). Ye S, Dhillon S, Ke X,et al.(2001) An efficient procedure forgenotyping single nucleotide polymorphisms. Nucleic AcidsRes 29:e88.